

Development and Validation of an Automated Thawing and Mixing Workcell,

Charles D. Hawker,^{1,2*} William L. Roberts,^{1,2} Antonio DaSilva,³ Gordon D. Stam,¹ William E. Owen,⁴ DeVirl Curtis,¹ Byung-Sang Choi,^{5†} and Terry A. Ring⁵ (¹ ARUP Laboratories, Salt Lake City, UT; ² Department of Pathology, School of Medicine, University of Utah, Salt Lake City, UT; ³ Motoman, Inc., Irvine, CA; ⁴ ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT; ⁵ Department of Chemical and Fuels Engineering, College of Engineering, University of Utah, Salt Lake City, UT; * address correspondence to this author at: ARUP Laboratories, 500 Chipeta Way, Salt Lake City, UT 84108; fax 801-584-5261, e-mail hawkercd@aruplab.com); [†] Current address: Department of Materials Science and Engineering, Gwangju Institute of Science and Technology (GIST), Republic of Korea)

Background: Working toward a goal of total laboratory automation, we are automating manual activities in our highest volume laboratory section. Because half of all specimens arriving in this laboratory section are frozen, we began by developing an automated workcell for thawing frozen specimens and mixing the thawed specimens to remove concentration gradients resulting from freezing and thawing.

Methods: We developed an initial robotic workcell that removed specimens from the transport system's conveyor, blew high-velocity room temperature air at the tubes, mixed them, and replaced them on the conveyor. Aliquots of citrated plasma were frozen with thermocouples immersed in the tubes, and thawing times and temperatures were monitored. Completeness of mixing of thawed specimens was studied by careful removal of small aliquots from the uppermost layer of the upright tubes without disturbing tube contents and analysis of total protein and electrolytes.

Results: High velocity ambient air aimed directly at tubes ranging from 12 × 75 to 16 × 100 mm brought specimens to room temperature in a maximum of 23 min. Adequate mixing of the specimens by the workcell's robot required only 2 approximate 126° move-

ments from an upright starting point, a surprising observation, because laboratorians are usually trained to mix 10 or 20 times. We also observed that, in a frozen overfilled tube, resulting analyte concentrations will be lower because more concentrated solutes leak from the tube.

Conclusions: A high-throughput, automated thawing and mixing workcell was successfully built, validated, and installed on our automated transport and sorting system.

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Our laboratory is a high-volume esoteric reference laboratory, accepting approximately 25–30 000 specimens per day. One of our largest laboratory sections, the Automated Core Laboratory, receives approximately 25% of that daily volume, performing more than 140 different chemistry, immunoassay, and specific protein tests, emphasizing cancer antigens, endocrine testing, and urine chemistry, but not routine serum chemistry. More than half of these 6–7000 specimens per day are frozen. The laboratory has been thawing them manually at room temperature (to prevent degradation of labile analytes) by blowing air from an ordinary electric fan at batches of tubes, a process requiring more than 1 h to assure complete thawing. The specimens were then mixed by manually inverting the tubes 10 times, before decapping for the various analyses.

Our long-term objective for this laboratory section is to interface analyzers to our automated transport and sorting system to achieve total laboratory automation and to automate other manual activities such as inspecting for adequate specimen volume and for the presence of interfering substances as indicated by hemolysis, lipemia, and icterus. The development of some form of automated workcell for rapidly thawing specimens at room temperature and mixing the thawed specimens was a 1st step toward our overall automation objective. We are not aware that such a robotic system has previously been built or described.

Our design intention with the workcell was to leave the specimen tubes in their track carriers (see Supplemental Fig. 1 that accompanies the online version of this Technical Brief at <http://www.clinchem.org/content/vol53/issue12>). Therefore, we sought to aim the thawing air directly at the tubes in the carrier through a slit normally used for reading bar codes. We developed an experimental apparatus with which we could evaluate a variety of nozzle designs that would direct air into the carrier slit (Supplemental Fig. 2). This design included thermocouples to monitor the thawing times of the tubes. Initially, thawing of frozen water was evaluated, but later, when the conditions of air flow and nozzle design had been established, we tested the system with specimens of out-of-date blood bank plasma. These studies (shown in Supplemental Fig. 3) indicated that a nozzle shaped as a small brass plug with a 2-mm orifice on a beveled edge (Supplemental Fig. 4) aimed at the bottom of the specimen

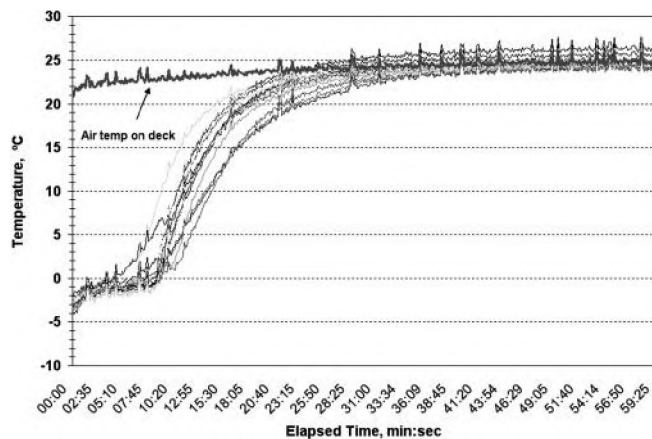


Fig. 1. Temperatures continuously monitored by thermocouples in tubes of frozen, out-of-date blood bank plasma in different locations on the deck of the workcell.

The temperatures in all tubes reached 20 °C in 24 min or less in this study at a variable frequency drive setting of 40% (24 cycles per second). The variable frequency drive setting chosen for routine operation was 45% (27 cycles per second), which gave slightly greater air flow, with all tubes at or above 20 °C in 23 min or less.

portion of the false-bottom tube would bring specimens frozen in dry ice to 20 °C in 22–23 min, at a plenum pressure of 8.795×10^4 Pa with an air flow of 0.000434 m³/s (0.434 L/s) per nozzle.

The Motoman workcell (Supplemental Fig. 5) uses an HP3XFC articulated 6-axis robot to remove specimen carriers from the track and place them on the deck in holders that prevent them from falling over, even with high velocity air blowing at them. A 10-hp, 480-volt, 60-cycle compressor directs the air into a plenum, which comprises the deck of the system. A total of 760 brass nozzles as described above are attached to the plenum, each designed to aim at the tube directly in front of it through the slit in the carrier. A variable frequency drive regulates compressor speed to maintain constant pressure in the plenum, thus optimizing the air flow from each nozzle. The number of nozzles in the deck design was based on the reach of the robot combined with a target workcell throughput speed of at least 1000 specimens per hour. The robot was fitted with a custom-designed pneu-

matic tool that grasps up to 10 carriers, with tubes, at a time for transfers between the conveyor system and deck and for mixing. This tool also uses pneumatic pin cylinders that press tightly against the top of each tube cap. In case a tube is capped with a push-top instead of a screw cap, the pin cylinders assure that the cap will not loosen during mixing and possibly leak.

The completed workcell was subjected to a rigorous validation study. In our thawing studies, most of the 760 locations on the thawing deck were populated with labeled tubes of frozen water, except that in key locations we placed plasma specimens in labeled tubes with temperature-recording thermocouples and surrounded those tubes with other plasma specimens in labeled tubes. The intent was to have a full deck of 760 frozen specimens to determine their impact on the plenum temperature and the overall thawing rate. In repetitive studies we then adjusted the air flow from the compressor by varying the compressor speed, seeking to balance an optimal thawing time against generation of excess heat and compressor noise. Compressing air generates heat (1), and, at higher compressor speeds (higher air flow), the plenum became warmer than desired for thawing of specimens, as determined by baseline temperature measurements. The results of one of these thawing studies with different air flows are shown in Fig. 1. All of the thermocouple temperature readings were above 20 °C in 23 min or less. These rapid thawing times were achieved with a combination of high air flow (0.000333 m³/s, or 0.0333 L/s, per specimen) and directing the air flow into the slit of the carrier so that it wrapped around the tube, thawing it from all sides. We postulated that, without the transport carrier, the air would simply have deflected off the front of the tube, and the back of the tube would not have thawed as quickly. This air flow was obtained with the variable frequency drive set at 45%, which delivered an alternating current frequency of 27 cycles per second.

The formation of concentration gradients in serum, urine, and other frozen specimens after the specimens are thawed is known (2, 3), and thawed specimens must be well mixed before they can be analyzed. Most laboratorians are trained to mix specimens 10–20 times before testing, but we were unable to find a published reference

Table 1. Summary of analytical results on the upper layers of thawed specimens after indicated number of mixes (n = 5 for each group).

No. of Mixes	Sodium, mmol/L		Potassium, mmol/L		Chloride, mmol/L		Albumin, g/L	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Baseline	167.6	0.55	3.50	0.05	75.0	0.00	38.0	0.00
0	129.4 ^a	12.46	2.70 ^a	0.26	58.0 ^a	5.83	29.2 ^a	0.26
2	167.0	0.00	3.40	0.09	75.0	0.00	38.0	0.00
4	167.8	1.10	3.40	0.04	75.0	0.45	37.8	0.04
6	167.8	0.45	3.50	0.05	75.2	0.45	37.8	0.04
8	168.0	1.00	3.40	0.08	75.6	0.55	37.8	0.04
10	168.2	0.45	3.50	0.05	75.2	0.45	38.3	0.05
12	167.8	0.84	3.40	0.05	75.4	0.04	38.2	0.04

^a Significantly different from baseline value by paired t-test, $P < 0.005$; all other P values > 0.05 .

recommending any particular number of mixes. We designed the workcell's 6-axis robot to perform the mixing by maintaining the specimens over a fixed location, so that a stainless steel pan could be positioned to catch drips from any leaking specimens. This design, coupled with the design of the pneumatic tool and its pin cylinders limited the range of rotation of the tubes to 126° in either direction. The resulting mixing pattern consisted of a rotation of approximately 126° to the left from an upright starting position, return to upright, then 126° to the right, and return to upright. A single 126° tilt and return to upright constitutes 1 mix cycle in this discussion.

We evaluated 0, 2, 4, up to 12 mix cycles each with 5 different replicate expired blood bank plasma specimens of 4.5 mL using our standard false bottom tube. The tubes were thawed on the workcell deck, but without the air blowing, to avoid any vibration or shaking of the tubes. After the tubes had thawed, we carefully sampled 200 μ L from the uppermost layer of each tube. The 1st set of replicates was sampled without robotic mixing, the 2nd set was sampled after 2 mix cycles, the 3rd set after 4 mix cycles, and so on. The aliquots were analyzed for albumin, sodium, potassium, and chloride on a Modular P analyzer (Roche Diagnostics) and compared to 5 replicates of unfrozen plasma that served as baseline or expected values. The results are shown in Table 1. After only 2 mix cycles (2 elevations to 126° followed by return to upright), the levels of all 4 analytes were indistinguishable from the baseline levels. This result was surprisingly fewer than we had expected based on experience. However, because human mixing motions may not duplicate the uniform speed and angles of our programmed robot (approximately 2 seconds to tilt 126° and return to upright), we are not recommending that laboratorians reduce their specimen mixes.

Knowing that an air bubble was required to achieve specimen mixing, we evaluated overfilling tubes with plasma in an attempt to determine the minimum size of air bubble necessary for adequate mixing. The volume of water expands approximately 9% when it is frozen (4). Furthermore, if a tube is filled too full and leaks due to this expansion, specimen solutes (minerals, proteins, etc.) preferentially squeeze through the cap threads, as has been reported for frozen, overfilled standard solutions (5) and for frozen, overfilled serum and urine specimens (2), and leaked specimens will be unacceptable for testing because the concentrations of analytes will have changed. We also learned that the minimum size of air bubble to facilitate mixing was 1.0 mL, which was sufficiently below the top of the tube to prevent leakage during freezing.

In summary, we designed, validated, and installed an automated thawing and mixing workcell, which is connected to our automated transport system and has a throughput to thaw and mix >1000 specimens per hour. The 6-axis robot appears able to effectively mix specimens with fewer mixes than routinely taught to laboratorians. Overfilled specimens that leak when frozen are unacceptable for laboratory analysis.

Grant/funding support: This work was supported by ARUP Laboratories.

Financial disclosures: None declared.

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Previously published online at DOI: 10.1373/clinchem.2007.094185
